Rh Complexity

Serology and DNA genotyping

Connie M. Westhoff, SBB, PhD
Scientific Director, American Red Cross, and
Adjunct, University of Pennsylvania,
Department of Pathology and Laboratory Medicine

American Red Cross

Molecular Blood Group
and Platelet Testing Laboratory

Rh typing

U.S.A.- American Red Cross referral experience

- Why serologic D typing is not always straightforward
  - C and e typing also
- Summarize problems
  - How they present
  - Clinical implications
  - Examples
- Perspective – role for genotyping in transfusion medicine
  - Current
  - Future

American Red Cross

Molecular Blood Group
and Platelet Testing Laboratory

Why is D typing sometimes problematic ?

In U.S.- Large number of variables in serologic testing

1. **Multiple Methods** – Hospitals - 91% tube test, 1% Solid phase, 8% Gel
   Some perform AHG test for weak D, others do not
   Donor Centers - Automated analyzers (Olympus PK), tube tests

2. **Different Reagents** – Contain different clones
   Can react differently with weak or variant D antigens
   FDA - only reactivity with DIV, DVa, and DVI need be specified
   Results in D typing discrepancies

3. **Variability in expression of RhD protein** (**~120** different genes = variations)
   All due to changes at the DNA level from "conventional" sequence

Weak D	-	53 different mutations
Partial D	~ 45	" "
D_{el}	~ 8	" "
"others"	~ 18	

Variation in expression of RhD

D Positive - Majority are "conventional"

1. Weak D - previously "Du"
   - **incidence- 0.2-1% - wide population differences**
   - **requires indirect antiglobulin test to detect (depends on reagent)**

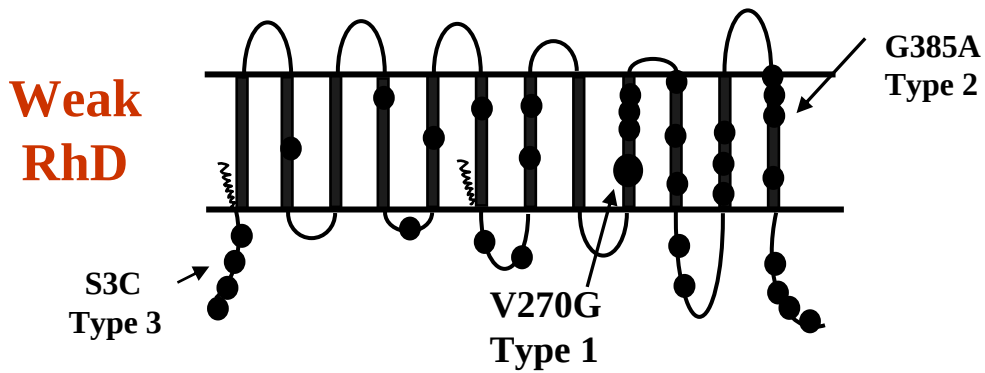
2. D_{el} - **very weak expression of D**
   - "**el" because adsorb and elute anti-D**

3. Partial D - previously called "mosaic"
   - **"missing" part of RhD**
   - **type as D-positive**
   - **make anti-D**

4. D epitopes - **expressed on Rhce proteins**
   - **cause D typing discrepancies**
     D^{HAR}, Crawford, ceRT, ceSL

Weak D (Wagner et al. Blood 93:385, 1999)

-single gene mutations – intracellular or cytoplasmic
-many different weak D (Type 1 thru 53 as circles)
- effect quantity of protein, but not D epitopes
usually do not make anti-D



Reactivity of weak D is variable with different monoclonal antibodies and with different techniques

many 3+ , but some very weak +/- or missed in IAT

Our experience. - weak D type 2, but by Gel testing stronger

D_{el}

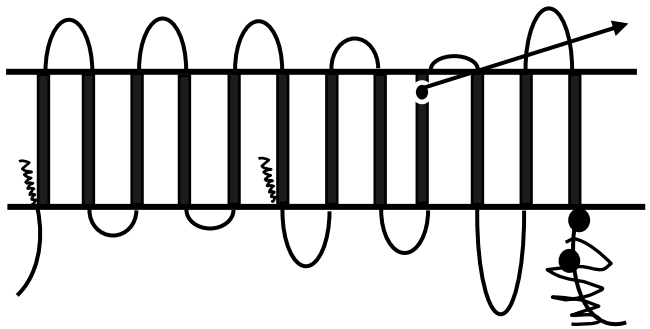
– type as D negative, (including IAT)

- adsorb and elute anti-D

8 different mutations

1/3 of Asians who type D negative; are also C+

In Caucasians - are C+ or E+



Recently "in the news" - have stimulated anti-D in recipients

- most would agree should be D positive as donors
- C or E serologic screening would eliminated from donor pool

Partial D

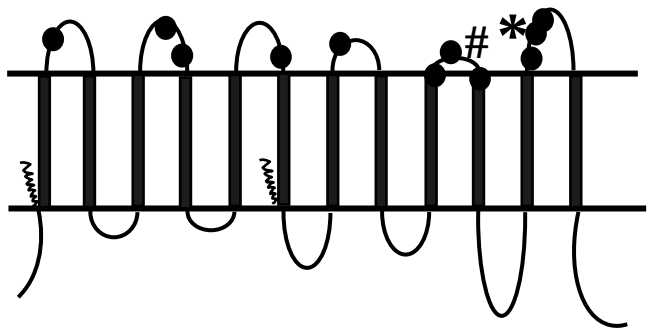
– **type as D positive, make anti-D**

- don't detect until make antibody

- mutations located on the extracellular surface

altered D epitopes -

some are due to single mutations

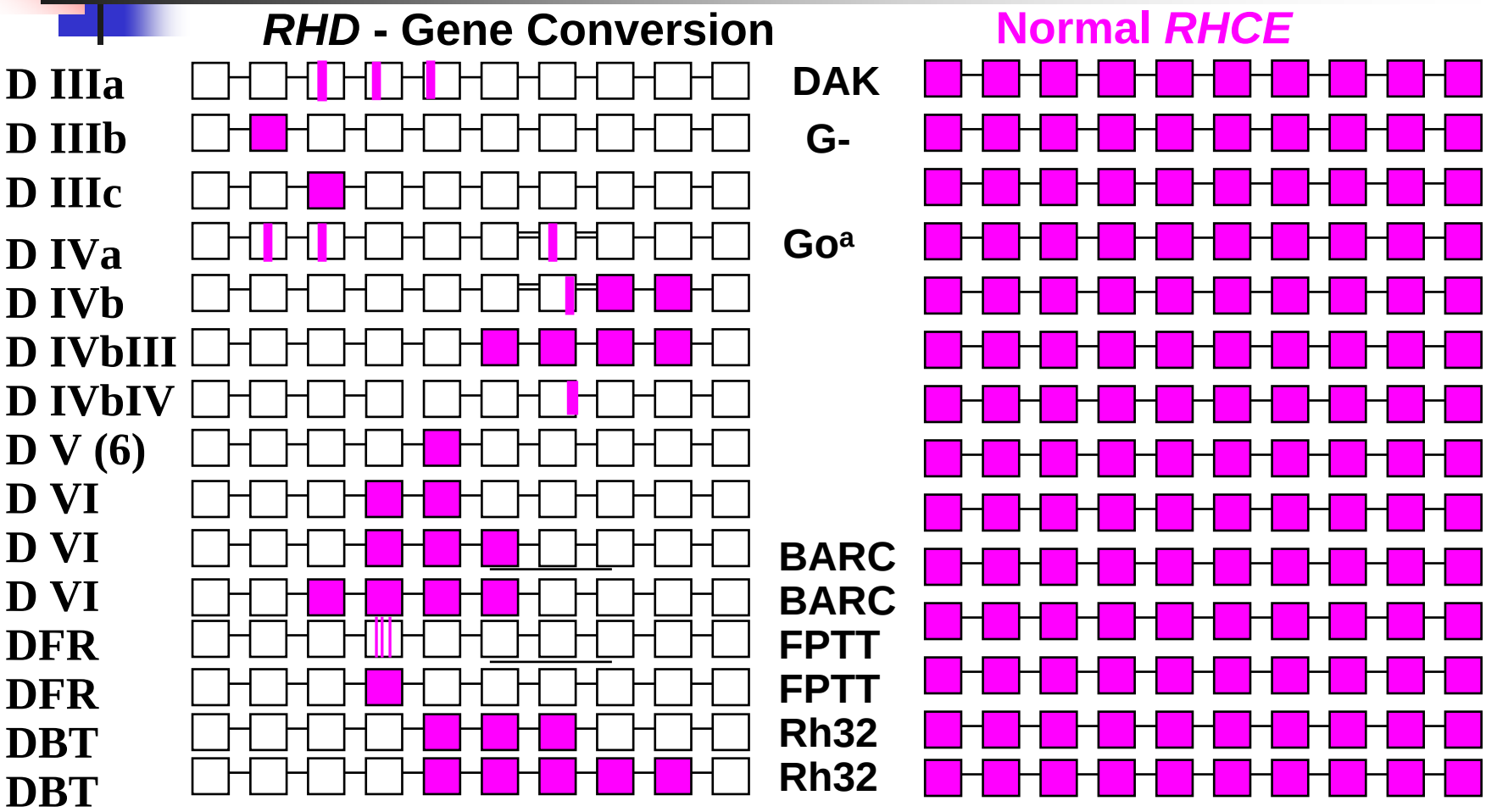


DMH - L54P
DVII - L110P
DFW - H166P
DHR - R229K
DHO - K235T

#weak D type 15 - G282D
D^{HMI} - T283I
DIM - C285Y
DNU - G353R
D^{II} - A354R

***DNB – G355S- more common in Europe**

Most Partial D's - *RHD* replaced with *RHCE*



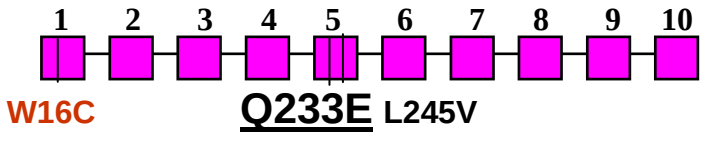
Alter D epitopes and create new antigens

D epitopes expressed on Rhce

RHD

No RHD GENE

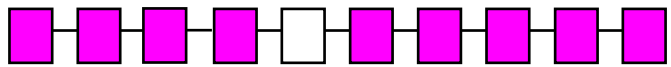
RHCE



Crawford

Blacks

No RHD GENE



D exon

R_o^{HAR} – D^{HAR}

Caucasian – German

D-specific amino acid (s) in the Rhce protein

strong reactivity with some monoclonal anti-D

In the US these are a major cause of D typing discrepancies that are referred for *RHD* gene investigation

FDA licensed reagents in use in U.S.

4 reagents for Tube testing, 1 for gel

REAGENT	IgM monoclonal	IgG
Gammaclone	**GAMA401**	**F8D8 monoclonal**
Immucor Series 4	**MS201**	**MS26 monoclonal**
Immucor Series 5	**Th28**	**MS26 monoclonal**
Ortho BioClone	**MAD2**	**Human-polyclonal**
Ortho Gel (ID-MTS)	**MS201**	

Only two contain same IgM clone

Clones can differ in reactivity with variant D antigens

Difference in reactivity of D^{Har} and Crawford+ RBCs with FDA licensed reagents

REAGENT	IgM monoclonal	IgG	D^{Har}	Crawford
Gammaclone	**GAMA401**	**F8D8 monoclonal**	POS	**POS**
Immucor Series 4	**MS201**	**MS26 monoclonal**	POS	NEG
Immucor Series 5	**Th28**	**MS26 monoclonal**	POS	NEG
Ortho BioClone	**MAD2**	**Human-polyclonal**	**NEG**	NEG
Ortho Gel (ID-MTS)	**MS201**		POS	NEG

D^{Har} and Crawford+ RBCs are non-reactive with human source polyclonal anti-D

Has contributed to perception in U.S. that D typing discrepancies are greater with monoclonal reagents

Case

81 year old African-American woman

2003- typed as D positive (3+ IS) - received 3 units

6/22/2006- admission for anemia, GI bleed

D positive, antibody screen negative

received 3 units D positive blood

7/4/2006 - strongly +DAT, low hgb, elevated bilirubin

Delayed transfusion reaction

Eluate and serum: Anti-D

Case

Anti-D	IS	RT	AHG
Gamma *(DMB40-2)*	**3+**	**4+**	**NT**
Gamma *(DMB36-1)*	**3+**	**3+^{s}**	**NT**
Gamma *(D139-2)*	**3+**	**3+**	**0**
Immucor Series 4	**0**	**0**	**0**
Immucor Series 5	**0**	**0**	**0**
Ortho Bioclone	**0**	**0**	**0**

Gamma IgM clone – strongly D positive

Ortho and Immucor clones - D negative, weak D neg

D typing "recommendations"

As patients/recipients – should be D negative

		D^{HAR}	Crawford	
Gammaclone	GAMA401	POS	POS	Donor reagent
Immucor Series	MS201	POS	NEG	
Immucor Series	TH28	POS (varies)	NEG	
Ortho BioClone	MAD2	NEG	NEG	Patient reagent
Ortho Gel Card	MS201	POS	NEG	

How often are these encountered ?

Estimated that Crawford- 1:900 AA in Southern US

D^{Har} ? German background - higher in Midwest

Our experience – Crawford represent large number samples referred for D typing discrepancies

Summary of *RHD* referrals

2006 (Jan - Sept) 9 months/ 210 samples

RHD	num
D discrepancies	
Crawford	15
weak D type 2	7
type 18	3
type 1	2
type 15	1
D_{el} (M295I)	1
Partial DAU-2	1
	30
D+ with anti-D	
DAR	3
DIIIa	2
Crawford	2
DIVa type 1	3
DIVb	1
DVI	1
weak D type 2	2
	14
***RHD* zygosity**	16

Serologic D typing
For Donor Testing

Goal: to label all donor RBCs with D antigen as D positive

Problem:

Weak D - **some are missed - even with IAT testing**

D_{el} - **all are typed as D negative**

These can stimulate anti-D in D negative patients

Important: all D_{el} (to date) and majority of weak D are inherited with C+ or E+, so can be removed from the D negative donor pool by serologic typing for C and E.

Questions not yet answered:

- **Will we accept no anti-D ?**
- **In specific group - girls and women of child bearing age**
- **Accept anti-K (HDFN) and anti-c in U.S., so inconsistent ?**

Serologic D typing
For patient and OB testing

Goal: to detect those at risk for anti-D

"Most" weak D- **are not at risk for anti-D (there are exceptions)**

Partial D **– at risk for anti-D, but type as D positive so are not detected**

- female children & women of child-bearing age better served treated as D negative for transfusion and RhIG candidates

Problems:

- Serologic tests cannot distinguish weak D from partial D
- Weak D mutations may also alter D epitopes

DNA genotyping for *RHD*

Would this resolve the "D problem" ?

- More complex than change in methodology

- How to act on the results ?

Variant D (weak and partial)- if treat all as D negative- would be significant burden to D negative donor pool

- Additional data needed -which *RHD* change/generate new epitopes ?

What is the Goal ? **To have no anti-D produce in any patient ?**

High throughput platforms needed:

- Many regions of gene must be sampled
- Complex algorithm for interpretation

Summary of case referrals

2006 (Jan - Sept) 9 months/ 210 samples

RHD	num
D discrepancies	
Crawford	15
weak D type 2	7
type 18	3
type 1	2
type 15	1
D_{el} (M295I)	1
Partial DAU-2	1
	30
D+ with anti-D	
DAR	3
DIIIa	1
DIVa type 1	4
DIVb	1
DVI	1
weak D type 2	2
	14
RHD zygosity	16

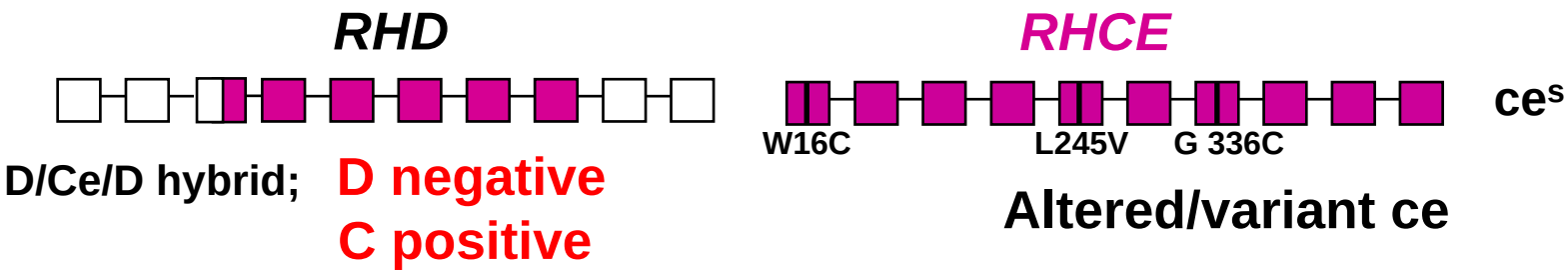
RHCE	num
e+ variants (most with anti-e)	**43**
C+ variants (with anti-C or -Ce	**8**
e discrepancies	6
E discrepancies	2

Many RHCE problems also

Others	0f 210 samples
ABO discrepancies	14
Dombrock screening	30
Typing multiply transfused pts.	14
Duffy typing discrepancies	3
McLeod	4
MNSs, U-	13
Misc. Nulls/new polymorphisms	6

When is serologic C typing not straightforward ?

In African Americans, Hispanic, and mixed ethnic groups with a specific *RHD-CE (3-8)-D* hybrid gene



Type as C positive; make anti-C

Hybrid *D-CE-D* gene is linked to variant e; make anti-e

This RH haplotype is prevalent in sickle cell patients

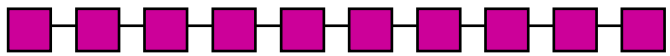
estimated 22% African Americans

American Red Cross

Molecular Blood Group and Platelet Testing Laboratory

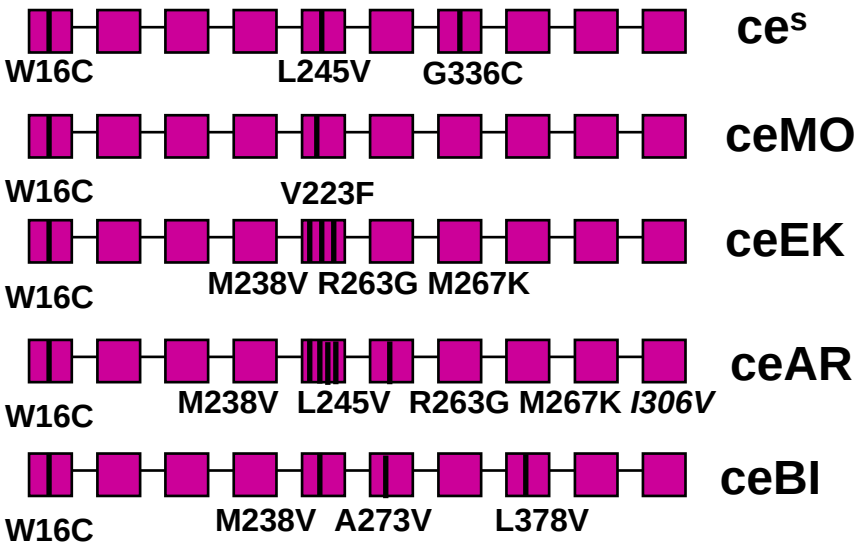
When is serologic e typing not straightforward ?

In African Americans, Hispanic, and mixed ethnic groups



“conventional” ce

Many different genes, all encode altered expression of e antigen



Type as e positive

Make anti-e and/or anti-ce

Prevalent in sickle cell patients

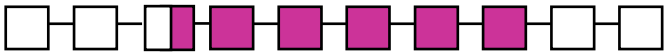
American Red Cross

Molecular Blood Group and Platelet Testing Laboratory

When is serologic e typing not straightforward ?

In African Americans, Hispanic, and mixed ethnic groups

associated with altered C

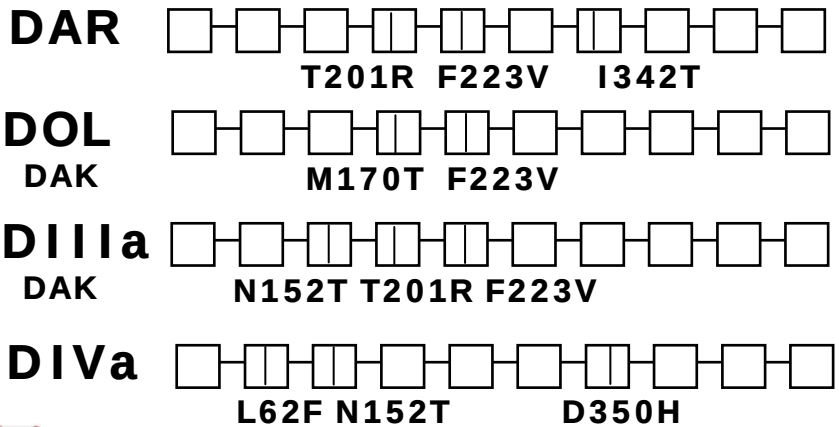


D/Ce/D hybrid

Type as C positive

Make anti-C

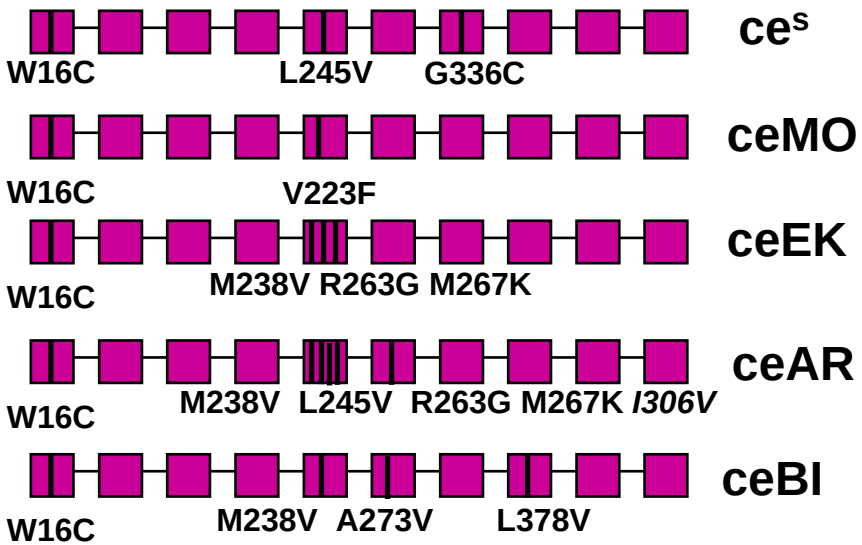
associated with altered D



Type as D positive

Make anti-D

altered expression of e



Type as e positive

Make anti-e and/or anti-ce

Prevalent in sickle cell patients

American Red Cross

Molecular Blood Group and Platelet Testing Laboratory

Role for genotyping in transfusion medicine

Current:

- Type multiply transfused patients
- Screen donor units when reagents are not available
- Resolve reagent typing discrepancies
  - Reveal shortcomings of current reagents- important to design of future reagents
- Predict fetal risk for hemolytic disease
  - Paternal RHD zygosity
  - Genotype fetus- amino fluid or maternal plasma
- Resolve antibody identification (is it allo or auto)
- Provide compatible donor units for sensitization sickle cell patients (variant D, C, and e antigens)

Role for genotyping in transfusion medicine

Going Forward:

Donors: Screen for antigen negative units with high throughput

- 2 types
  - One serologic
  - One DNA based

Will provide the opportunity to validate genotyping and to detect any exceptions in different populations

Patients: Sickle cell “antigen matching” programs

- **Transfusion predicted to increase with STOP trial outcome**
  - **Variant C, e, and D makes “genetic match” for Rh superior**
  - **Detect those at risk for production of antibodies to high-incidence Rh antigens.**

Role for genotyping in transfusion medicine

Future:

Integration of DNA-based assays into blood bank

- as genomics is applied to diagnosis and treatment

- Potential for “antigen” matching
  - How many antigens (15 or 50 ?)
  - Which patient population
    - Those needing long term transfusion support
    - For all
- At what cost? Or…..at what savings?
  - Impact workload
    - Walk-away systems
    - Decrease sensitization=decreased ABID

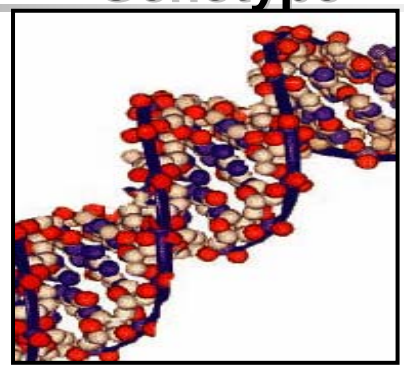
Require major changes in management of donor inventory

Blood group “typing”

power in combination of both technologies

Phenotype **Genotype**

+



agglutination **DNA**

Focus should not be

to prove or decide if one method is superior OR
to replace all serologic typing with DNA-based testing

Focus

use each to strengthen the other

Shortcoming of D typing reagent revealed by DNA testing
– use knowledge to improve serologic reagents

Shortcomings of SNP testing revealed by serology
- use knowledge to design additional SNP

Acknowledgements

- Molecular Blood Group and Platelet Testing Laboratory
  - Sunitha Vege
  - Wynn Meyer
  - Michelle Henry
  - Trina Whorley
  - Cindy Marks

Support - **National Reference Laboratory Blood Group Serology**
  - Sandra Nance
  - Staff

American Red Cross